1103326-018

#19
JRP
2/15/96

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : C. Carling, et al.

Serial No. : 08/317,407

Filed : October 3, 1994

For : COMBINATION OF A BRONCHODILATOR AND STEROIDAL ANTI-INFLAMMATORY DRUG FOR THE TREATMENT OF RESPIRATORY DISORDERS, AS WELL AS ITS USE AND THE PREPARATION THEREOF

DECLARATION UNDER 37 C.F.R. § 1.132

I, Jan William Trofast, Ph.D., declare as follows:

I am Principal Research Scientist in Pharmaceutical and Analytical Research and Development at Astra Draco AB in Lund, Sweden, a subsidiary of Astra AB, the assignee of the above-identified application. My curriculum vitae is attached as Exhibit A.

I am a coinventor of the subject matter of the above-identified patent application, and I participated in the August 17, 1994 Examiner interview. I am familiar with the office actions issued during the course of prosecution of this application and its parent, as well as the prior art patents of Brattsand, et al. and Murakami, et al. cited against the pending claims.

0000AUQW.W51

A Declaration under 37 C.F.R. § 1.132, signed by me on May 24, 1995, was submitted on June 9, 1995 as a follow-up to Applicants' May 23, 1995 Amendment and Response. In the subsequent office action of August 30, 1995, the Examiner asserted that the data set forth in the Declaration are not sufficient to demonstrate nonobviousness over the cited prior art because they are not commensurate in scope with the invention as claimed. In response to the Examiner's assertion, the pharmacological *in vivo* studies set forth below were performed; they were carried out at my behest by pharmacologists at Astra Draco AB.

The data set forth in the previous Declaration demonstrated the synergistic potency of combinations of formoterol and budesonide in molar ratios ranging from 1:1 to 1:20 in reducing lung inflammation. The new data presented herein were obtained from tests in which a formoterol-budesonide combination in a 1:60 molar ratio of the two active ingredients was administered. The new tests were performed using the same Sephadex-induced edema model in rats according to the protocols set forth in the May 24, 1995 Declaration. The new results are presented in Table I below:

TABLE I

Inhibition of Sephadex-Induced Lung Inflammation in Rats

Compound	Amount Administered (nmol/kg)	n†	% inhibition
1. Budesonide	120	6	21
2. Formoterol + Budesonide (1:60)	2 + 120	6	69* (p=0.0104)

† number of animals subjected to regimen

Statistical parameters: * $p<0.05$; ** $p<0.01$

The inhibition of inflammation by formoterol administered alone at 2 nmol/kg was taken to be 13%, the value previously determined in tests of 12 animals and set forth in the May 24, 1995 Declaration.

As in the previously performed tests with formoterol-budesonide combinations, the statistical analysis was obtained by comparing the effect of the given combination with the effect of the corresponding amount of budesonide administered without formoterol. This type of analysis was designed to particularly point up enhanced anti-inflammatory effects of the combination in comparison to the effects expected (and observed) for the anti-inflammatory steroid (budesonide) component alone.

In the previously performed tests, it was observed that budesonide by itself in a concentration range from 5 nmol/kg to 40 nmol/kg produced no significant inhibition of Sephadex-induced edema in the rat. From Table I above it can be seen that even a dosage of 120 nmol/kg of budesonide alone gave only 21% inhibition of inflammation; this is insignificant compared to that observed with administration of a placebo. As before, however, the effect of formoterol and budesonide in combination (69% inhibition) was seen unquestionably to be significant ($p=0.0104$), based on the criterion of the Wilcoxon rank sum test, and far greater than the sum of the individual effects of each of the components.

It is known in the pharmacology art that rat cells exhibit 3-10 times greater sensitivity to glucocorticosteroids than does man, although this could differ to some extent in *in vivo* test models. The work of Claman, New England J. Med. 287, 388-397 (1972), copy attached as Exhibit B, is representative of the knowledge in the field. Particular attention is called to Table 2 of Claman and the text under "SPECIES DIFFERENCES" on pages 388-389.

The effect of administration of a 1:60 molar ratio of formoterol to budesonide in the instant rat test regimen, then, can be taken to be reflective of the effect

of a 1:200 or lower molar ratio of the two active ingredients administered to a human subject.

It should be emphasized that it would be difficult to demonstrate a statistically significant synergistic effect of, for example, an actual 1:200 ratio of formoterol to budesonide in the rat (as opposed to the 1:60 ratio in the rat which is reflective of a 1:200 ratio in a human); because of the heightened sensitivity of the rat, budesonide at such a high dosage would have a significant effect by itself. Conversely, if one were to take into account the heightened sensitivity of the rat by decreasing the dosage of formoterol in order to achieve the lower formoterol-to-budesonide ratio, the resultant formoterol dosage would be too low to have an effect, not just alone but even in combination with budesonide; the lower limit of activity must always be considered in administration of a therapeutic agent.

The data herein provide further demonstration that budesonide-formoterol combinations over a wide range of molar ratios provide an enhancement of anti-inflammatory effect which, unexpectedly, is significantly greater than the sum of the individual anti-inflammatory effects of the two active agents. More precisely, the data herein, as well as those set forth in the previous Declaration,

demonstrate that neither budesonide without formoterol nor formoterol without budesonide provide significant reduction of Sephadex-induced inflammation at the administered concentrations. On the other hand, treatment of animals with the combined agents in the same concentrations administered individually resulted in unquestionable, significant reduction of inflammation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: June 19 Dec. 1995

Jan W. Trofast

JAN W. TROFAST, Ph.D.